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Research paper

A rapid two dot filter assay for the detection of *E. coli* O157 in water samples

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ABSTRACT

E. coli O157:H7 is an enterohemorrhagic bacteria that cause deadly water-borne infections implicated in outbreaks of a wide spectrum of human gastrointestinal diseases. It is therefore important to have a rapid convenient, simple and sensitive range of detection of *E. coli* O157:H7. A new *E. coli* O157 MAb designated P124 was developed for ultrasensitive detection of *E. coli* O157 in water, apple juice and beef for routine use. A prototype filter dot assay was designed with anti-*E. coli* O157 MAb bound to 0.2 μm nitrocellulose filter disk as the capture antibody. A 100 ml water sample spiked with 1–50 CFU of *E. coli* O157 either in the presence or absence of other non-specific bacteria were filtered for capture of the pathogen on the antibody coated nitrocellulose disk. The detection of the pathogen was successfully accomplished by the same antibody both as a capture and detecting antibody as a homosandwich. In a non-enriched format, detection of *E. coli* was possible with a sensitivity of 2500 CFU/100 ml. Ultrasensitive detection of ~1 CFU/100 ml sample could be achieved by a prior pathogen enrichment step before the addition of the labeled antibody. The design of this diagnostic test is based on the common architecture of all bacteria, viruses and spores, namely the manifestation of repeat lipopolysaccharide epitopes on the surface. We have developed an easy-to-use two dot visual filter assay for translation into current water testing in public health laboratories to detect *E. coli* O157:H7. In a 5 h assay ~1 CFU and ~5 CFU of *E. coli* O157 could be detected in 100 ml of water or juice and lake samples respectively. This simple homosandwich enrichment strategy can also be used to detect low levels of other water-borne pathogens.

1. Introduction

E. coli O157:H7 causes a wide spectrum of human diseases, including bloody and non-bloody diarrhea, hemorrhagic colitis, occasional kidney failure, hemolytic uremic syndrome (HUS) and death at times (DeCludt et al., 2000; Shelton and Karns, 2001) due to ingestion of meat (Willshaw et al., 1994), water, and uncooked fruits and vegetables (Pebody et al., 1999). Outbreak of *E. coli* O157:H7 infections through drinking water was first reported in the USA in 1989 (Swerdlow et al., 1992). O157 contamination of drinking and recreational water has emerged as important cause of human disease (Ackman et al., 1997; Armstrong et al., 1996; Chalmers et al., 2000; Friedman et al., 1999). *E. coli* O157 present in the drinking water offered to livestock contributed to the prevalence of infection in animals leading to the contamination of meat products and the environment (Elder et al., 2000). An extreme example of the dangers this bacteria poses was seen in the Walkerton, Ontario and in the neighboring Maritimes, described as Canada's worst-ever outbreak of *E. coli* contamination (Hrudrey et al., 2003). *E. coli* O157: H7 is a top disease concern of the multi-billion dollar North American cattle industry. In Canada, Alberta has the greatest population of cattle and hence live stock generated manure contamination of water from surface drainage channels has precipitated one of the highest levels of gastroenteritis resulting from *E. coli* O157:H7 and *Salmonella* species (Khakhria et al., 1996). *E. coli* O157:H7 is the most common strain of Shiga toxin producing enterohemorrhagic

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E. coli (STEC) in United States, Canada and United Kingdom (Kaper, 1998). Several recent outbreaks of gastrointestinal diseases caused by STEC has highlighted the threat these organisms pose to public health and are also considered as potential biowarfare agents (Trochimchuk et al., 2003).

Many detection methods have been employed to rapidly detect low levels of pathogens in food, beverages and water. Current techniques include traditional enrichment and plating methods with selective media such as Sorbitol MacConkey agar and Rainbow agar (Manafi and Kremsmaier, 2001; Meng et al., 2001; Novicki et al., 2000). *E. coli* O157 is particularly difficult to confirm from enrichment cultures, even with highly selective media due to the problem of high background levels of competing microorganisms including other type of serotypes of *E. coli.* A variety of immunological methods have been developed for the detection and enumeration of *E. coli* O157 whole bacteria (Chapman et al., 1991, 1997; Park and Durst, 1999; Todd et al., 1988). The common denominator among all methods was the use of monoclonal or polyclonal anti-*E. coli* O157 antibodies to selectively capture, or capture and label by sandwich assay of *E. coli* O157 whole bacteria. Enrichments and plating often take 24–48 h to identify the organism. It is therefore important that rapid sensitive methods are developed to detect *E. coli* O157 during outbreaks, surveillance and quality control to prevent costly errors and fatalities due to delayed detection. Hence we have developed a simple 5 h two dot assay on nitrocellulose filter disks using a growth medium. This enrichment allows viable bacteria to grow for a short period prior to detection in a visual immunoassay (Fig. 1).

2. Materials and methods

2.1. Bacterial strains

Strains used for the development of two dot filter assay were obtained from Dr. Roger Johnson's group (Health Canada Labs, Winnipeg, Canada) and from Dr. Newman & Edie Ashton (Provincial lab of Alberta). The strains of bacteria include *E. coli* O157:H7 (ATCC 43895), *E. coli* non-O157: non-H7 (O6: H34, O26:H11), *E. coli* non-O157: H7 (O18:H7, O91:H7), *Salmonella urbana, S. typhimurium*, *Pseudomonas aeruginosa* and 5 other laboratory strains of *E. coli* namely, JM87, Top 10 F, BL21DE3, JM109 and MKH14. All the strains used for the experiments were grown on Trypticase soy agar plates at 37 °C overnight followed by growth in Trypticase soy broth (TSB, pH 7.2–7.3) for 18 h with gentle agitation at 200 rpm. The classical dilution and colony counting methods were supplemented with direct estimation of fluorescent bacterial counts for accurate bacterial concentration measurements as described previously by our group (Guttikonda et al., 2004).

2.2. P124 MAb conjugation with colloidal gold

A 10 ml aliquot of 20 nm colloidal gold (Sigma–Aldrich St. Louis, MO, USA) was adjusted to pH 9.0 with 0.1 M K_2CO_3. The P124 MAb at 10 μg/ml was adjusted to pH 9.0 with 100 nM K_2CO_3 and 10 ml of the antibody solution was added to 10 ml of colloidal gold. The solutions were shaken gently for 15 min at RT. Subsequently, 1 ml of 10% BSA pH 9.0 was

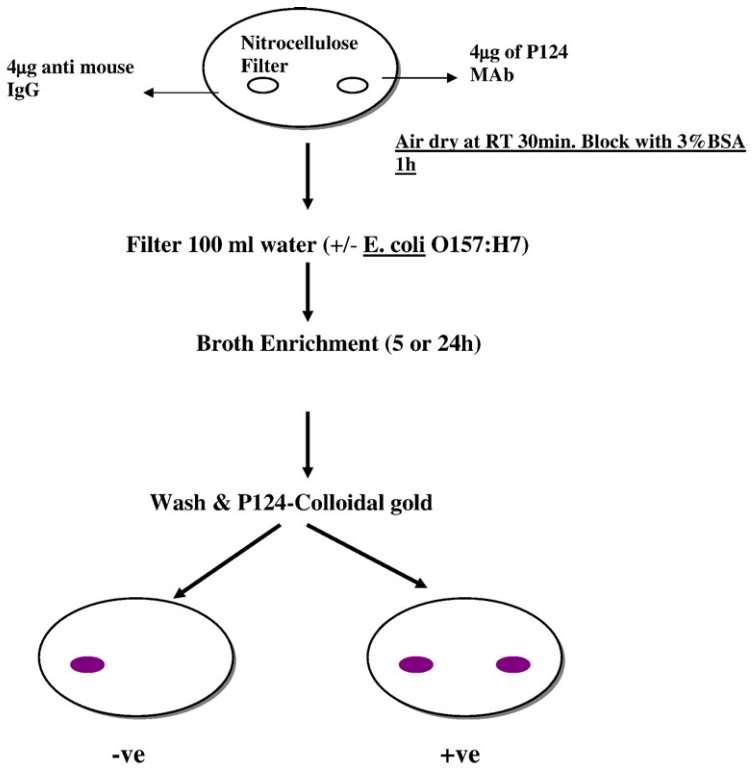

Fig. 1. Diagrammatic representation of our two dot filter assay for the detection of *E. coli* O157:H7.